

Claims

- 1           1. Recombinant enzymatically active  
2 glucocerebrosidase produced by a eukaryotic cell.
- 1           2. The glucocerebrosidase of claim 1, produced  
2 by an insect cell.
- 1           3. The glucocerebrosidase of claim 1, produced  
2 by a mammalian cell.
- 1           4. The glucocerebrosidase of claim 3, produced  
2 by a Chinese hamster ovary cell.
- 1           5. Recombinant enzymatically active  
2 glucocerebrosidase comprising at least one exposed  
3 mannose residue, said glucocerebrosidase being capable  
4 of binding with a human mannose receptor protein.
- 1           6. The recombinant enzymatically active  
2 glucocerebrosidase of claim 1 or 5, wherein said  
3 glucocerebrosidase has an amino acid sequence with at  
4 least 95% homology to an amino acid sequence of a  
5 primate glucocerebrosidase.
- 1           7. The recombinant enzymatically active  
2 glucocerebrosidase of claim 6, wherein said primate  
3 glucocerebrosidase is human glucocerebrosidase.
- 1           8. The recombinant enzymatically active  
2 glucocerebrosidase of claim 5, comprising at least two  
3 exposed mannose residues.
- 1           9. The recombinant enzymatically active  
2 glucocerebrosidase of claim 8, comprising a carbohydrate  
3 moiety having between 3 and 9 exposed mannose residues.

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1           10. The recombinant enzymatically active  
2 glucocerebrosidase of claim 9, wherein said between 3  
3 and 9 mannose residues are arranged in a  $\text{Man}_3$  to  
4  $\text{Man}_9$  structure.

1           11. The recombinant enzymatically active  
2 glucocerebrosidase of claim 5, wherein said receptor  
3 protein is a human mannose receptor protein occurring  
4 naturally in a phagocytic cell.

1           12. The recombinant enzymatically active  
2 glucocerebrosidase of claim 5, wherein said  
3 glucocerebrosidase is produced within an insect cell.

1           13. The recombinant enzymatically active  
2 glucocerebrosidase of claim 5, wherein said  
3 glucocerebrosidase is produced within a mammalian cell.

1           14. A eukaryotic cell comprising nucleic acid  
2 encoding enzymatically active glucocerebrosidase,  
3 wherein said glucocerebrosidase is capable of  
4 specifically binding with a human mannose receptor  
5 protein.

1           15. The eukaryotic cell of claim 14, said cell  
2 being an insect cell.

1           16. The eukaryotic cell of claim 14, said cell  
2 being a mammalian cell.

1           17. The eukaryotic cell of claim 16, said  
2 mammalian cell being a Chinese hamster ovary cell.

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1           18. The eukaryotic cell of claim 14, wherein  
2 said nucleic acid comprises DNA encoding human  
3 glucocerebrosidase.

1           19. The eukaryotic cell of claim 14, wherein  
2 said DNA lacks at least 50% of a naturally occurring  
3 region between the promoter of said  
4 glucocerebrosidase-encoding DNA and the ATG start site  
5 of said glucocerebrosidase-encoding DNA.

1           20. The eukaryotic cell of claim 19, wherein  
2 said cell is an insect cell.

1           21. The eukaryotic cell of claim 19, wherein  
2 said cell is a mammalian cell.

1           22. The eukaryotic cell of claim 21, said  
2 mammalian cell being a Chinese hamster ovary cell.

1           23. The insect cell of claim 15, wherein said  
2 nucleic acid is provided by pVL941.GCRD21.

1           24. The insect cell of claim 15, wherein said  
2 nucleic acid is provided by a vector comprising DNA  
3 encoding an amino acid sequence having at least 95%  
4 homology to an amino acid sequence of a naturally  
5 occurring glucocerebrosidase.

1           25. The insect cell of claim 24, wherein said  
2 nucleic acid is provided by pAc373.GCR2.2.

1           26. The insect cell of claim 24, wherein said  
2 naturally occurring glucocerebrosidase occurs naturally  
3 within a primate.

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1           27. The insect cell of claim 26, wherein said  
2 naturally occurring glucocerebrosidase occurs naturally  
3 within a human.

1           28. The eukaryotic cell of claim 14, wherein  
2 said glucocerebrosidase comprises at least two exposed  
3 mannose residues.

1           29. The eukaryotic cell of claim 28, wherein  
2 said glucocerebrosidase comprises a carbohydrate moiety  
3 having between 3 and 9 mannose residues.

1           30. The eukaryotic cell of claim 29, wherein  
2 said between 3 and 9 mannose residues are arranged in a  
3 Man<sub>3</sub> to Man<sub>9</sub> structure.

1           31. An insect comprising a cell of claim 15.

1           32. A mammal comprising a cell of claim 16.

1           33. A method for producing enzymatically  
2 active glucocerebrosidase comprising the steps of:  
3           introducing nucleic acid encoding  
4 glucocerebrosidase into a eukaryotic cell;  
5           causing said cell to express said  
6 glucocerebrosidase; and  
7           purifying said glucocerebrosidase.

1           34. The method of claim 33 wherein said  
2 eukaryotic cell is an insect cell.

1           35. The method of claim 33 wherein said  
2 eukaryotic cell is a mammalian cell.

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1           36. The method of claim 35 wherein said  
2 mammalian cell is a CHO cell.

1           37. The method of claim 33 wherein the step of  
2 causing said cell to express said glucocerebrosidase  
3 comprises culturing said cell in a culture medium in  
4 vitro.

1           38. The method of claim 34 wherein the step of  
2 causing said cell to express said glucocerebrosidase  
3 comprises growing said cell in vivo within an insect.


1           39. The method of claim 35 wherein the step of  
2 causing said cell to express said glucocerebrosidase  
3 comprises growing said cell in vivo within a mammal.

1           40. The method of claim 37 wherein the step of  
2 purifying said glucocerebrosidase comprises purifying  
3 said glucocerebrosidase from said culture medium.

1           41. The method of claim 33 wherein the step of  
2 purifying said glucocerebrosidase comprises disrupting  
3 said cell to form a cellular extract and purifying said  
4 glucocerebrosidase from said cellular extract.

1           42. The mammalian cell of claim 16 wherein  
2 said cell is transformed with any plasmid selected from  
3 the group pGB20, pGB37, and pGB42.

1           43. The mammalian cell of claim 16 wherein  
2 said cell is cotransformed with plasmid pGB34 and any  
3 plasmid selected from the group pGB20, pGB37, and pGB42.



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1           44. The method of claim 37 wherein the pH of  
2 said culture medium is between about pH 6.5 and pH 7.2.

1           45. The method of claim 44 wherein the pH of  
2 said culture medium is between about pH 6.6 and pH 6.8.

1           46. The method of claim 37 wherein said  
2 culture medium contains O<sub>2</sub> in an amount below about  
3 50% saturation and sufficient to maintain the cells.

1           47. The method of claim 37 wherein said  
2 culture medium contains O<sub>2</sub> in an amount between about  
3 20% saturation and about 30% saturation.